

Screening of Methanol Extract and Ethyl Acetate Fraction of *Abies webbiana* Lindl. for Neuropharmacological Activities

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Parkash, *et al.*: Neuropharmacological Activities of *Abies webbiana*

Despite a long traditional use of *Abies webbiana* Lindl. (Talispatra; family-Pinaceae) in the treatment of mental disorders, the plant has not been investigated systematically to validate its traditional claims. Thus, the present investigation was undertaken with an objective to investigate neuropharmacological activities of methanol extract of *Abies webbiana* aerial parts and its ethyl acetate fraction. Properly identified aerial parts were defatted with petroleum ether and then extracted with methanol in a Soxhlet apparatus. Ethyl acetate fraction was prepared by partitioning methanol extract with ethyl acetate using standard procedure. In acute toxicity study, no mortality was observed in animals after oral administration of 2 g/kg dose of methanol extract. The methanol extract (200 or 400 mg/kg, *p.o.*) and ethyl acetate fraction (25 or 50 mg/kg, *p.o.*) were evaluated for antianxiety, anticonvulsant, antidepressant, sedative, antistress and analgesic activities using well established models. The methanol extract and ethyl acetate fraction of *Abies webbiana* aerial parts exhibited significant antianxiety, anticonvulsant, antidepressant, sedative, antistress and analgesic activities with respect to control. Preliminary phytochemical screening showed presence of flavonoids in bioactive ethyl acetate fraction of *Abies webbiana* aerial parts. It is finally concluded that flavonoids are the bioactive constituents responsible for most of neuropharmacological activities of *Abies webbiana*.

Key words: Anxiolytic, antidepressant, analgesic, pinaceae, sedative, talispatra

Mental disorders include anxiety, depression, stress, seizures, schizophrenia and hyperactivity^[1,2] are common to all countries, and people suffering with these disorders are often subject to social isolation, poor quality of life and increased mortality^[3]. About 14% of the global burden of disease has been attributed to mental disorders^[4]. Although synthetic drugs are easily available in market for the treatment of mental disorders but these drugs are associated with severe side effects such as memory impairment, addiction and dependence^[5], sexual dysfunction^[6], headaches, dizziness and abnormal vision^[7]. Researchers are exploring natural resources to find out newer, efficacious and safer drugs. Traditional plants seem viable alternatives for the development of newer and safer psychotropic drugs^[8]. *Abies webbiana* is one of such plants which has long tradition of use in the treatment of mental disorders.

Abies webbiana Lindl. commonly known as Talispatra, belongs to family Pinaceae. The plant is

distributed throughout India, mostly in the Himalayan region from Kashmir to Assam states. The plant is also abundant in Afghanistan, Tibet, Nepal and Bhutan at an altitude of 2500-4000 m^[9]. The plant has been traditionally used in the treatment of various ailments such as mental disorders, rheumatism, bronchitis, pulmonary infections, carminative, antiseptic, asthma, expectorant, stomachic, cough and decongestant^[10-14].

Despite a long tradition of use in neurological disorders, no systematic pharmacological work has ever been carried out on *A. webbiana* to validate its traditional claims. Therefore, it was considered

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worthwhile to evaluate methanol extract and ethyl acetate fraction of *A. webbiana* aerial parts for various neuropharmacological activities.

MATERIALS AND METHODS

A. webbiana aerial parts were procured from D. G. Ayurvedic Sangrah, Andheri, Mumbai, India in September, 2013. The plant was identified from Department of Botany, Punjabi University, Patiala. A herbarium-cum voucher specimen has been deposited to the Department of Botany, Punjabi University, Patiala. Petroleum ether (60-80°, RFCL Ltd., New Delhi, India), ethyl acetate (E Merck, Delhi, India) and methanol (S.D. Fine Chem., Mumbai, India), of LR grade were employed in present studies.

Preparation of extract and fraction:

Dried powdered aerial parts of *A. webbiana* (1 kg) were defatted by extracting exhaustively with petroleum ether in a Soxhlet apparatus. The marc was then extracted with methanol in similar manner. The methanol extract (10 g) of *A. webbiana* aerial parts was suspended uniformly in water and partitioned with ethyl acetate by refluxing for 30 min at 50° along with continuous stirring. This procedure of partitioning with ethyl acetate was repeated for ten times. All the separated layers of ethyl acetate were pooled and concentrated under reduced pressure to get ethyl acetate fraction (EF). Methanol extract and EF were subjected to phytochemical screening^[15].

Animals:

Laca mice (either sex) of body weight 20-25 g purchased from the Central Research Institute, Kasauli, India were used for pharmacological and acute toxicological studies. The animals were fed with normal laboratory pellet diet and water *ad libitum*. The approval was taken from Institutional Animal Ethics Committee of Punjabi University, Patiala before carrying out animal studies (107/99/CPCSEA/2013-22, dated 14/09/2013). The animals were acclimatized to laboratory conditions daily for 1 h for continuous seven days before the start of experiment. All the experiments were carried out from 9 AM to 12 PM as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals. Groups of six mice were used in all sets of experiments. The animals were overnight fasted before use. The doses were administered orally with the help of an oral cannula fitted on a tuberculin syringe.

Vehicle and standard drugs:

Distilled water+Tween 80 (2%) was used as vehicle for preparing various test doses of test samples in such a concentration as to administer a volume ranging 0.2 to 0.25 ml to the mice. Diazepam (Triko Pharmaceuticals, Rohtak, Haryana), thiopentone sodium injection (Neon Laboratories Ltd., Mumbai, India), phenytoin sodium injection (Magnet Labs Pvt. Ltd., New Delhi, India), imipramine (Triko Pharmaceuticals, Rohtak, Haryana) and morphine sulphate injection (Verve Health Care Ltd., Gujrat, India) were used as standard drugs. Diazepam (1 mg/kg, *i.p.*), diazepam (2 mg/kg, *i.p.*), thiopentone sodium injection (80 mg/kg, *i.p.*), phenytoin sodium injection (20 mg/kg, *i.p.*), imipramine (15 mg/kg, *i.p.*) and morphine sulphate injection (5 mg/kg, *i.p.*) were used as standard antistress, anxiolytic, sedative, anticonvulsant, antidepressant and analgesic drugs respectively.

Acute toxicity study:

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines-423^[16]. After oral administration of the methanol extract of *A. webbiana* aerial parts (2000 mg/kg/*p.o.*), animals were observed individually for behavioural profile (alertness, restlessness, irritability and fearfulness), neurological profile (spontaneous activity, reactivity, touch response and pain response) and autonomic profile (defecation and urination) for at least once during the first 30 min and periodically during the first 24 h, for a total of 14 days.

Experimental protocol:

Animals were divided into six (I-VI) groups. Group I - control group received vehicle (0.25 ml, *p.o.*); Group II - standard group received respective standard drug; Group III - test group received methanol extract (200 mg/kg, *p.o.*); Group IV - test group received methanol extract (400 mg/kg, *p.o.*); Group V - test group received EF (25 mg/kg, *p.o.*) and Group VI - test group received EF (50 mg/kg, *p.o.*).

Antianxiety activity using elevated plus maze model (EPM):

The plus-maze apparatus consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to observe anxiolytic behaviour in animals^[17]. During the 5 min experiment, the number of entries into the open arms and average time spent by the mouse in the open arms (average

time=total time spent in open arms/number of entries in arms) were recorded in mice.

Anticonvulsant activity using maximal electro shock test (MES):

An electroconvulsive shock was applied through ear-clip electrodes of electroconvulsometer (Rolex, Patiala, India) to induce tonic hind limb extension in mice^[18]. The maximal electroshock stimulus used for mice was 50 mA for 0.2 s. Time spent by the mice in tonic extensor phase of convulsions and percentage protection of animals were recorded.

Antidepressant activity using despair swim test:

Mice were forced to swim, after 1 h of administration of test substances, in a Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water upto the level of 15 cm, and maintained at $25\pm 2^{\circ}$ ^[18]. Mice were allowed to swim for 6 min, and the total duration of immobility was noted.

Sedative activity using thiopentone sodium induced sleeping assay:

After 30 min of administration of vehicle and test extracts, thiopentone sodium (80 mg/kg, *i.p.*) was injected to induce sleep in mice^[19]. The time taken for onset of sleep, and interval between loss and recovery of righting reflex were recorded.

Antistress activity using cold swimming test:

Mice were forced to swim, after 1 h of administration of test drugs, in a Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water upto the level of 15 cm, and maintained at $10\pm 2^{\circ}$ ^[18]. Mice were allowed to swim for 6 min, and the total duration of immobility was noted.

Analgesic activity using tail immersion test:

Groups of mice were subjected to noxious stimulus (radiant heat) by placing 5 cm of the tail in a 500 ml beaker containing 450 ml water maintained at $55\pm 2^{\circ}$ before and after treatment with test drugs^[18]. The reaction time at 30 min, 1, 2 and 3 h were recorded after the treatment. The percentage maximum possible effect (% MPE) is calculated from the formula, % MPE=(reaction time–basal time/Cut off time–basal time)×100.

Statistics:

The results have been expressed as mean±standard deviation (SD). The test drugs were compared with

standard drug and control by one way analysis of variance (ANOVA) followed by Student Newman Keul's test^[20].

RESULTS AND DISCUSSION

Literature survey has revealed that *A. webbiana* has a long tradition of use in the treatment of mental disorders, but no scientific work has ever been carried out to validate traditional claims of *A. webbiana*. Thus, the present investigations have been undertaken with an objective to generate neuropharmacological data on this medicinally promising plant.

The methanol extract of *A. webbiana* aerial parts was prepared by Soxhlation process, and yield was found to be 16.21% w/w. Preliminary phytochemical screening of methanol extract of *A. webbiana* aerial parts showed presence of flavonoids as one of the major groups of phytoconstituents. Thus, it was planned to separate flavonoid rich fraction from methanol extract by partitioning it with ethyl acetate applying standard procedure. The yield of EF was found to be 18.45% w/w calculated in relation to methanol extract.

Acute toxicity studies showed no mortality in mice after oral administration of 2 g/kg dose of the methanol extract of *A. webbiana* aerial parts. No lethality and toxic reactions were observed until the end of the study period, thus, the dose is said to be “unclassified” under the toxicity scale. Therefore, 200 and 400 mg/kg doses of methanol extract which are 1/10th and 1/5th of the dose tested for acute toxicity study respectively, were selected for detailed pharmacological activities evaluation. EF was found to be about 1/5th part of methanol extract, thus, 25 and 50 mg/kg doses were selected for pharmacological activities evaluation.

The methanol extract and EF of *A. webbiana* aerial parts were subjected to antianxiety activity in mice using EPM model. The mean number of entries and average time spent in open arms of EPM apparatus after the treatment of methanol extract (200 or 400 mg/kg, *p.o.*), EF (25 or 50 mg/kg, *p.o.*), diazepam (2 mg/kg, *i.p.*) and the control (vehicle, *p.o.*) in mice have been shown in Table 1. The methanol extract and EF exhibited significant antianxiety activity with respect to control at tested doses, but therapeutic level equivalent to the standard

drug was not achieved at lower doses. Higher doses, i.e., 400 and 50 mg/kg of the methanol extract and EF, respectively, significantly increased mean number of entries in open arms of EPM in animals not only with respect to control but also equivalent to the standard drug. It is clearly evident from Table 1 that, only methanol extract significantly increase mean time spent in open arms at 400 mg/kg, which was statistically comparable to the standard drug.

The methanol extract and EF of *A. webbiana* aerial parts were subjected to anticonvulsant activity in mice using MES test. The decreased duration of MES-induced tonic extensor phase in the mice and percentage protection of animals were noted after the treatment of methanol extract (200 or 400 mg/kg, *p.o.*), EF (25 or 50 mg/kg, *p.o.*), phenytoin (20 mg/kg, *i.p.*) and the control (vehicle, *p.o.*) as shown in Table 2. The methanol extract and EF exhibited significant anticonvulsant activity at all doses with respect to control, but the activity was not equivalent to the standard drug phenytoin. The standard drug completely abolished duration of tonic extensor phase in mice and protected all animals from MES-induced convulsions, where as methanol extract and EF reduced mean duration of tonic extensor phase to 16.48 and 15.01 s, respectively, with respect to control (20.18 s). These observations suggest mild anticonvulsant activity of *A. webbiana*.

The methanol extract and EF of *A. webbiana* aerial parts were subjected to antidepressant activity in mice using despair swim test. Table 3 shows the mean time spent by mice in immobile state after the treatment of methanol extract (200 or 400 mg/kg, *p.o.*), EF (25 or 50 mg/kg, *p.o.*), imipramine (15 mg/kg, *i.p.*) and the control (vehicle, *p.o.*). The methanol extract (400 mg/kg) and EF (50 mg/kg) exhibited significant antidepressant activity with respect to control as well as equivalent to the standard drug. Mice treated with lower doses of methanol extract (200 mg/kg) and EF (25 mg/kg) spent significantly higher time in immobile state as compared to the group of mice treated with the standard drugs.

The methanol extract and EF of *A. webbiana* aerial parts were subjected to sedative activity in mice using thiopentone sodium induced sleeping assay. The mean latency time and duration of sleep after administration of methanol extract (200 or 400 mg/kg, *p.o.*), EF (25 or 50 mg/kg,

TABLE 1: ANTIANXIETY ACTIVITY OF *ABIES WEBBIANA* USING EPM

Treatment	Dose (mg/kg)	Mean ⁿ number of entries in open arms±SD	Mean ⁿ time spent in open arms (s)±SD
Control	Vehicle	2.73±1.06 ^a	4.83±0.85 ^a
Standard drug (diazepam)	2	7.77±0.89*	11.32±1.22*
Methanol extract	200	5.75±0.96 ^{*,a}	8.35±0.84 ^{*,a}
	400	7.12±0.67*	10.62±1.41*
EF	25	5.43±1.21 ^{*,a}	8.07±0.71 ^{*,a}
	50	7.03±1.04*	9.47±1.15 ^{*,a}

n=6, the data is expressed as mean±SD. **P*<0.05 versus control, ^a*P*<0.05 versus standard, one-way ANOVA followed by Student Newman-Keul's test. SD: standard deviation, ANOVA: analysis of variance, EPM: elevated plus maze model, EF: ethyl acetate fraction

TABLE 2: ANTICONVULSANT ACTIVITY OF *ABIES WEBBIANA* USING MES TEST

Treatment	Dose (mg/kg)	Mean ⁿ time spent in extensor phase (s)±SD	Percentage of protection of animals
Control	Vehicle	20.18±0.06 ^a	50
Standard drug (phenytoin)	20	0±0*	100
Methanol extract	200	16.48±0.99 ^{*,a}	50
	400	15.64±1.02 ^{*,a}	66.66
EF	25	15.01±1.59 ^{*,a}	66.66
	50	14.56±1.56 ^{*,a}	83.33

n=6, the data is expressed as mean±SD. **P*<0.05 versus control, ^a*P*<0.05 versus standard, one-way ANOVA followed by Student Newman-Keul's test. SD: standard deviation, ANOVA: analysis of variance, MES: maximal electro shock test, EF: ethyl acetate fraction

TABLE 3: ANTIDEPRESSANT ACTIVITY OF *ABIES WEBBIANA* USING DESPAIR SWIM TEST

Treatment	Dose (mg/kg)	Mean ⁿ immobility time (s)±SD
Control	Vehicle	197.11±4.71 ^a
Standard drug (imipramine)	15	44.05±2.83*
Methanol extract	200	78.17±2.48 ^{*,a}
	400	43.51±2.67*
EF	25	74.05±3.74 ^{*,a}
	50	44.51±1.87*

n=6, the data is expressed as mean±SD. **P*<0.05 versus control, ^a*P*<0.05 versus standard, one-way ANOVA followed by Student Newman-Keul's test. SD: standard deviation, ANOVA: analysis of variance, EF: ethyl acetate fraction

p.o.), thiopentone sodium (80 mg/kg, *i.p.*) and the control (vehicle, *p.o.*) has been shown in Table 4. It is clearly evident from the Table 4 that the vehicle could not further potentiate duration of sleep induced by thiopentone sodium. The animals treated with vehicle+thiopentone sodium showed statistically equivalent mean duration of sleep as shown by the animals treated with thiopentone sodium alone, thus, the sedative activity was confirmed in test drugs by comparing with control. The methanol extract increased duration of sleep to 65.83 and 99.97 min at a dose of 200 and 400 mg/kg, respectively, with

respect to control (59.49 min), whereas EF increased sleeping time in mice to 77.95 and 104.83 min at the doses of 25 and 50 mg/kg, respectively.

The methanol extract and EF of *A. webbiana* aerial parts were subjected to antistress activity in mice using cold swim test. Table 5 shows the mean immobility time of the mice after administration of methanol extract (200 or 400 mg/kg, *p.o.*), EF (25 or 50 mg/kg, *p.o.*), diazepam (1 mg/kg, *i.p.*) and the control (vehicle, *p.o.*). The methanol extract significantly reduced mean time spent by mice in

TABLE 4: SEDATIVE ACTIVITY OF ABIES WEBBIANA USING THIOPENTONE SODIUM-INDUCED SLEEPING ASSAY

Treatment*	Dose (mg/kg)	Mean ⁿ ±SD	
		Mean latency time (min)	Mean duration of sleep (min)
Control	Vehicle	1.26±0.08	56.49±1.71
Standard drug (thiopentone sodium)	80	1.65±0.05	56.11±2.57
Methanol extract	200	1.35±0.65	65.83±2.82*
	400	1.45±0.75	99.97±3.45*
EF	25	1.30±0.98	77.95±4.12*
	50	1.45±0.45	104.83±3.21*

n=6, the data is expressed as mean±SD. **P*<0.05 versus control, one way ANOVA followed by Student Newman-Keul's test. Thiopentone sodium (80 mg/kg, *i.p.*) was injected intraperitoneally to all groups of mice treated with control, methanol extract and EF. SD: standard deviation, ANOVA: analysis of variance, EF: ethyl acetate fraction

TABLE 5: ANTISTRESS ACTIVITY OF ABIES WEBBIANA USING COLD SWIM TEST

Treatment	Dose (mg/kg)	Mean ⁿ immobility time(s)±SD
Control	Vehicle	164.66±6.06 ^a
Standard drug (diazepam)	1	41.33±5.64*
Methanol extract	200	66.29±4.54 ^{a,*}
	400	50.29±6.01 ^{a,*}
EF	25	56.01±6.51 ^{a,*}
	50	40.67±3.62*

n=6, the data is expressed as mean±SD. **P*<0.05 versus control, ^a*P*<0.05 versus standard, one-way ANOVA followed by Student Newman-Keul's test. SD: standard deviation, ANOVA: analysis of variance, EF: ethyl acetate fraction

TABLE 6: ANALGESIC ACTIVITY OF ABIES WEBBIANA USING TAIL IMMERSION TEST

Treatment	Dose (mg/kg)	Basal time (s)	Reaction time (s)				Percentage of MPE			
			Mean ⁿ ±SD							
			30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
Control	Vehicle	2.10±0.22	2.75±0.11 ^a	2.70±0.14 ^a	2.60±0.15 ^a	2.50±0.25 ^a	5.04	4.65	3.87	3.10
Standard drug (morphine)	5	3.00±0.75	10.07±0.47*	8.51±0.37*	6.36±0.36*	4.50±0.22*	58.91	45.91	28.00	12.50
Methanol extract	200	2.29±0.55	6.55±0.39 ^{a,*}	5.75±0.10 ^{a,*}	4.86±0.12 ^{a,*}	2.82±0.14 ^a	33.51	27.22	20.22	4.17
	400	2.65±0.40	7.72±0.47 ^{a,*}	6.71±0.22 ^{a,*}	5.31±0.33 ^{a,*}	2.80±0.15 ^a	41.05	32.87	21.53	1.21
EF	25	2.08±0.01	7.91±0.36 ^{a,*}	5.63±0.28 ^{a,*}	3.47±0.44 ^{a,*}	2.86±0.27 ^a	45.12	27.47	10.75	6.04
	50	2.13±0.09	8.55±0.44 ^{a,*}	7.53±0.30 ^{a,*}	6.45±0.37*	4.37±0.50*	49.88	41.95	33.57	18.32

n=6, the data is expressed as mean±SD. **P*<0.05 versus control, ^a*P*<0.05 versus standard, one-way ANOVA followed by Student Newman-Keul's test. SD: standard deviation, ANOVA: analysis of variance, EF: ethyl acetate fraction, MPE: maximum possible effect

immobile state in comparison to control. But both doses of extract could not achieve therapeutic level when compared to standard drug. On the other hand, EF significantly reduced mean time spent in immobile state at a dose of 50 mg/kg with respect to control as well as equivalent to the standard drug.

The methanol extract and EF of *A. webbiana* aerial parts were subjected to analgesic activity in mice using tail immersion method. The tail withdrawal from the heat (flicking response) was recorded in the mice after administration of methanol extract (200 or 400 mg/kg, *p.o.*), EF (25 or 50 mg/kg, *p.o.*) and morphine sulphate (5 mg/kg, *i.p.*). The results have been shown in Table 6. The methanol extract and EF exhibited significant analgesic activity at the tested dose with respect to control group during 2 h of study. But none of test doses of methanol extract could show analgesic effects comparable to the standard drug. It is clearly evident from Table 6 that the reaction time of mice treated with test and standard drugs decreased in time dependent manner. The standard drug showed significant analgesic activity with respect to control during 3 h of study. Though EF could not able to achieve therapeutic level equivalent to the standard drug, but at higher dose it retained analgesic activity for 3 h similar to standard drug.

Preliminary phytochemical studies showed presence of flavonoids as major groups of phytoconstituents in methanol extract and EF of *A. webbiana* aerial parts. The available literature reveals that a large number of flavonoids such as rutin^[21], quercetin^[22], naringenin^[23], chrysin^[24], apigenin^[25], linarin^[26] and goodyerin^[27] have been reported to exhibit varied neuropharmacological activities through various mechanisms such as regulation of gamma-amino butyric acid type A (GABA_A) receptor, the chloride ion channel complex and 5-hydroxytryptamine_{1A} (5HT_{1A}) regulation,

monoamine oxidase inhibition and increasing availability of serotonin and noradrenaline in the synaptic cleft. In agreement to these reports, it is suggested from our results that neuropharmacological activities of *A. webbiana* aerial parts are attributed to flavonoids.

The authors are involved in isolating bioactive constituents from EF of *A. webbiana* following bioactivity-guided fractionation.

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Conflicts of interest:

There are no conflicts of interest.

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